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Award Number: DAMD17-00-1-0439

TITLE: Characterization of a Putative Tumor Suppressor in Breast Cancer

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REPORT DATE: June 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

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20040720 023

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 2003		3. REPORT TYPE AND DATES COVERED Annual Summary (1 Jun 2000 - 31 May 2003)
4. TITLE AND SUBTITLE  Characterization of a Putative Tumor Suppressor in Breast Cancer			5. FUNDING NUMBERS  DAMD17-00-1-0439	
6. AUTHOR(S)  Jackie Thomas, Ph.D. Michael A. White, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Southwestern Medical Center at Dallas Dallas, TX 75390-9105  E-Mail: Jackie.swanik@utsouthwestern.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES  The original contains color plates: ALL DTIC reproductions will be in black and white				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words)  Telomerase activity is required to maintain telomere integrity on chromosomes of proliferating cells and thus is critically involved in regulating cellular replicative lifespan. Telomerase is repressed in most adult somatic cells, and activation of telomerase activity is an early event associated with tumor progression. Expression of telomerase is sufficient to greatly prolong proliferative lifespan of human cells in culture. Because telomerase activity is not required to maintain viability of post-mitotic somatic cells, but is required to maintain the proliferative capacity of tumor cells, telomerase is an ideal target for anti-cancer therapies. Here we have produced mammalian expression vectors containing Pol-driven short-hairpin siRNA precursors targeting hTERT mRNA. We show that these vectors dramatically repress telomerase activity when delivered to telomerase positive immortal human tumor cells, resulting in dramatic telomere shortening and a limited replicative life-span in culture.				
14. SUBJECT TERMS  Breast Cancer			15. NUMBER OF PAGES 6	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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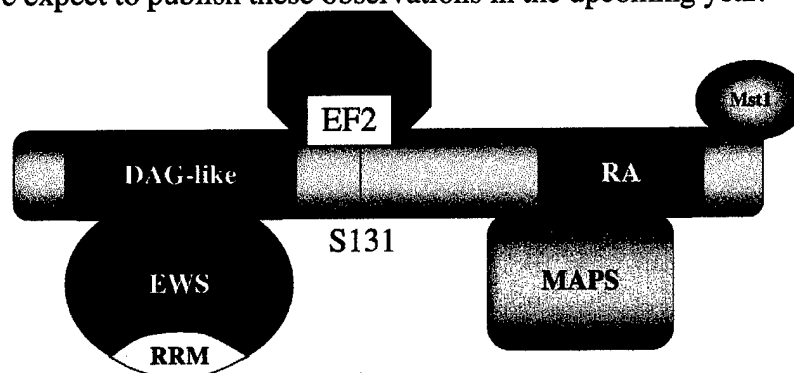
## Introduction:

The subject of this research is the RASSF1 locus located at 3p21. Our group and others have found that this locus expresses two related gene products, RASSF1A and RASSF1C. RASSF1A expression is lost at high frequency in a wide range of tumor types, primarily through hyper methylation of the RASSF1A-specific promoter. The purpose of this work was to explore the possibility that RASSF1A protein has important tumor suppressor activity. We employed gain-of-function and loss-of-function analysis to examine the contribution of RASSF1A to tumor cell growth and survival.

## Body:

Task 1: Analysis of functional consequences of RASSF1A and RASSF1C expression in breast cancer derived cell lines. As described in the previous report, this task has been completed (Burbee et al., 2001). We found that RASSF1A but not RASSF1C can revert tumorigenic phenotypes.

Task 2: To characterize the molecular physiology of RASSF1A. As described in the previous report, we have found that RASSF1A is a physiologically relevant negative regulator of cell cycle progression and this likely accounts for its tumor-suppressive activity (Shivakumar et al., 2002). We have demonstrated that RASSF1A inhibits cell cycle progression through inhibition of Cyclin D1 translation, and we have moved on to characterize the molecular connection between RASSF1A and this activity. During the past year we have identified and validated several RASSF1A-interacting proteins that establish links between RASSF1A and cyclin D1 translational machinery. These physical interactions suggest that RASSF1A can establish translational dormancy on cyclin D1 mRNA by assembling an inhibitory complex consisting of the RNA-binding protein EWS, and a novel EFG homolog that lacks a GTPase domain. We expect to publish these observations in the upcoming year.



Using a combination of yeast two-hybrid screens and validation by co-immunoprecipitation from HeLa cells and in vitro binding assays, we have found that RASSF1A interacts with EWS, MST1/2, and a novel EF2 homolog. We have begun a functional evaluation of these interactions. To date we find that inhibiting MST1/2 expression by RNAi will rescue RASSF1A-induced cell cycle arrest. In addition we have isolated several peptide aptamers that associate with RASSF1A in a manner that can inhibit MST1/2 interaction. We are currently testing the capacity of these aptamers to inhibit RASSF1A function in cells. Interestingly, it has recently been reported that RASSF1A expression is commonly inactivated in pediatric tumors, with the exception of Ewing's sarcoma. Given our observation of an EWS/RASSF1A complex, this presents an exciting genetic correlation that may indicate EWS may

function directly downstream of RASSF1A. We are evaluating this hypothesis by testing the consequences of EWS siRNA on RASSF1A-induced cell cycle arrest in H1299 cells. In addition, we have acquired several Ewing's sarcoma-derived cell lines. We will test the sensitivity of these lines to RASSF1A expression.

**Key Training Accomplishments:**

- Ms. Thomas has acquired technical expertise in tumorigenicity assays, protein-interaction assays, immunofluorescence analysis, and RNAi.
- Ms. Thomas has received training in hypothesis generation, hypothesis testing, and data acquisition and analysis.
- Ms. Thomas has learned the central importance of appropriate controls in the process of producing interpretable results.

**Key Research accomplishments:**

- Restoration of RASSF1A expression can revert tumorigenic phenotypes.
- RASSF1A negatively regulates Cyclin D1 translation.
- RASSF1A assembles a translational inhibitory complex on cyclin D1 mRNA.
- RASSF1A interacts with EWS.
- MST1/2 are required for the cytostatic activity of RASSF1A.

### **Reportable outcomes:**

Burbee, D. G., Forgacs, E., Zochbauer-Muller, S., Shivakumar, L., Fong, K., Gao, B., Randle, D., Kondo, M., Virmani, A., Bader, S., *et al.* (2001). Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J Natl Cancer Inst* 93, 691-699.

Shivakumar, L., Minna, J., Sakamaki, T., Pestell, R., and White, M. A. (2002). The RASSF1A Tumor Suppressor Blocks Cell Cycle Progression and Inhibits Cyclin D1 Accumulation. *Mol Cell Biol* 22, 4309-4318.

Shivakumar, L. (2002). RASF1A: A frequently targeted tumor suppressor that negatively regulates cell cycle progression. Ph.D. thesis. UT Southwestern Medical Center.

As a result of the training provided by this grant, Dr. Shivakumar is currently a Science Fellow at Aurigene Discovery Technologies.

### **Conclusions:**

This work strongly suggests that RASSF1A is a tumor suppressor protein that is frequently lost in human cancer. Our observation of germline mutations that result in expression of defective RASSF1A proteins has the potential to aid future risk assessment. Continued work directed at defining the molecular nature of RASSF1A function will enhance our understanding of breast cancer development and progression, and may reveal novel entry points for therapeutic strategies.

### **References:**

Burbee, D. G., Forgacs, E., Zochbauer-Muller, S., Shivakumar, L., Fong, K., Gao, B., Randle, D., Kondo, M., Virmani, A., Bader, S., *et al.* (2001). Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J Natl Cancer Inst* 93, 691-699.

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